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Chapter 7

Summary, translational aspects and future perspectives

SUMMARY

Significant advancements have been made in the elucidation of the pathogenetic pathways underlying atherosclerosis and glomerulosclerosis. However, a definite preventative or curative treatment option does not exist for these pathologies. Dysfunction of endothelial cells is a critical initiating process in both the pathogenesis of glomerulosclerosis and atherosclerosis. Aberrant gene and protein expression patterns lie at the basis of endothelial dysfunction and the concomitant phenotypic changes of endothelial cells. Aberrant epigenetic, transcriptional, and translational regulation of genes cause aberrant gene expression patterns and therefore underlies the development of endothelial dysfunction during pathogenesis. To halt the development and progression of glomerulosclerosis and atherosclerosis, and to improve the currently limited effective treatment options for both pathologic manifestations, a better understanding of differential epigenetic, transcriptional and translational regulation involved in endothelial dysfunction is needed. The overall aim of this thesis was to identify epigenetic, transcriptional and translational mechanisms involved in the modulation of the endothelial gene transcription underlying endothelial dysfunction in glomerulosclerosis and atherosclerosis. The specific aims of this thesis were to study and to pharmacologically modulate:

- the epigenetic enzyme Enhancer of Zeste homolog 2 (EZH2) and its concomitant histone modification trimethylation of lysine 27 of histone 3 (H3K27me3) in the regulation of the endothelial transcription involved in the loss of the endothelial glycocalyx in glomerulosclerosis
- the transcription factor peroxisome proliferator-activated receptor gamma (PPARɣ) in the induction of proteinuria via transcriptional regulation of the heparan sulphate degrading- enzyme heparanase
- the reciprocal cross-talk between Mitogen-Activated Protein Kinase 7 (MAPK7) and EZH2 in endothelial cells in the process of endothelial-to-mesenchymal transition (EndMT) in atherosclerosis

Glomerular endothelial cells (GEnCs), covered by an endothelial glycocalyx, form the glomerular capillaries and the first cellular barrier of the glomerular filtration barrier (GFB). GEnC are in an interdependent relationship with podocytes and mesangial cells and involves bidirectional cross-talk between both cell types [1]. In **chapter 2**, we reviewed literature that shows that 1) GEnC dysfunction occurs in early stages of glomerular sclerotic diseases including Focal Segmental Glomerulosclerosis (FSGS) and diabetic nephropathy (DN), 2) GEnC dysfunction precedes and contributes to podocyte

injury and contributes to mesangial activation, and 3) GEnC dysfunction is sufficient to develop proteinuria. GEnC dysfunction is characterized by a loss of fenestrae, a reduced endothelial glycocalyx thickness, a pro-inflammatory state, mitochondrial damage and oxidative stress, aberrant signalling and EndMT [2-15]. Aberrant gene expression largely contributes to GEnC dysfunction in glomerular sclerotic diseases. GEnC dysfunction results in proteinuria, podocyte damage or loss, mesangial activation, and ultimately glomerulosclerosis. Given the importance of GEnCs in the initial pathogenetic process, the glomerular endothelium poses a potential efficacious cellular target to pharmacologically halt disease development and progression in FSGS and DN.

The thickness of the glomerular endothelial glycocalyx is reduced in DN [10, 16]. A reduced glycocalyx thickness is associated with proteinuria and kidney failure [5, 17]. In **chapter 3**, we examined whether the epigenetic enzyme Enhancer of Zeste Homolog 2 (EZH2), that catalyzes trimethylation on lysine 27 of histone 3 (H3K27me3), inhibits the production of the glomerular endothelial glycocalyx and thereby reduces its thickness in DN. We show that EZH2 modulates the expression of endothelial glycocalyx-associated genes, and that a reduction in EZH2 expression and the accompanied reduction in H3K27me3 presence increases the thickness of the endothelial glycocalyx *in vitro*. Especially the expression of genes encoding glycosaminoglycan (GAG) polymerization proteins was increased after downregulating EZH2 expression. The level of H3K27me3 is increased in GEnCs of diabetic $BTBR^{ob/ob}$ mice compared to non-diabetic $BTBR^{wt/wt}$ mice, which was associated with a reduction in binding of the lectin LEA to GEnCs, which suggests a reduction in glycocalyx abundance. We were able to translate these experimental observations also into the clinical setting as an increase in H3K27me3 presence in GEnCs and a reduction of the glomerular endothelial glycocalyx in kidney biopsies from patients with DN were observed. This study therefore demonstrates that EZH2-mediated trimethylation of H3K27 silences the expression of glycocalyx-associated genes and is most likely responsible for the observed reduced glomerular endothelial glycocalyx in both experimental (mice) and clinical (human) DN.

In **chapter 4,** we evaluated whether the downregulation of (glomerular) endothelial EZH2 (resulting in reduced levels of H3K27me3) can be achieved *in vivo* and, if so, whether this results in ameliorated DN. An endothelial cell-specific siRNA-based targeting approach using liposomes containing the cationic lipid SAINT, i.e. SAINT-O-Somes [SOS], has previously shown to effectively deliver siRNA into activated endothelial cells in the kidney and to decrease locally the target mRNA expression and to prevent disease progression [18, 19]. To decrease EZH2 and H3K27me3 in GEnC, SOS loaded with siRNA against EZH2 were administered to diabetic $BTBR^{ob/ob}$ mice. *In vivo*, anti-E-selectin conjugated

SOS loaded with siEZH2 (siEZH2-SOS) were successfully delivered to GEnCs in BTBR$^{ob/ob}$ mice. SiEZH2-SOS treatment reduced glomerular collagen 1 expression and preserved glomerular perfusion area, whereas plasma creatinine was increased and creatinine clearance was reduced. A reduction in GEnC EZH2 expression could not be shown, but siEZH2-SOS treatment showed a trend towards decreasing H3K27me3 presence in GEnC compared to control siSCR-SOS treatment. No differences were observed in the abundance of the endothelial glycocalyx between siEZH2-SOS-treated mice and siSCR-SOS-treated mice. The results on EZH2 expression and H3K27me3 presence raise the question whether siEZH2-SOS treatment was sufficiently effective to decrease EZH2 and H3K27me3. Potentially, the timing of analysis of EZH2 and H3K27me3 after siEZH2-SOS treatment might have been suboptimal, resulting in a lack of a subsequent observable decrease in EZH2 and H3K7me3. Based on these *in vivo* data, it is still elusive whether reduced EZH2 in GEnC is beneficial in glomerulosclerosis in DN, despite reduced glomerular collagen I expression and preserved glomerular perfusion area. Whether reduction of EZH2 in GEnC is beneficial in glomerulosclerosis needs to be investigated in future research.

Glomerular expression of heparanase (HPSE), a key glycocalyx-degrading enzyme, is increased in many glomerular diseases and associates with the development of proteinuria [5, 17, 20, 21]. In **chapter 5,** we investigated whether the expression of HPSE is transcriptionally regulated by the transcription factor PPARɣ and whether this pathway is involved in the development of proteinuria. We therefore evaluated whether the PPARɣ agonist pioglitazone reduces the expression of HPSE at the transcriptional level and ameliorates proteinuria in an experimental rat model of adriamycin-induced nephropathy (AN) in which FSGS develops. Adriamycin increased glomerular HPSE expression, and was associated with reduced glomerular heparan sulphate (HS) expression and proteinuria. Treatment with the PPARɣ agonist pioglitazone normalized glomerular heparanase and HS expression in AN, indicating that PPARɣ inhibits glomerular heparanase expression. Conversely, in healthy rats, the PPARɣ antagonist GW9662 induced glomerular heparanase expression and reduced glomerular HS expression, which was associated with the development of proteinuria. *In vitro*, PPARɣ regulated the expression of heparanase in cultured podocytes. In line with the induction of proteinuria *in vivo*, the PPARɣ antagonist GW9662 induced transendothelial albumin passage in a heparanase-dependent manner. We showed that PPARɣ directly binds to HPSE promoter regions in podocytes and GEnC. Furthermore, the PPARɣ antagonist GW9662 induced heparanase promoter activity, suggesting that the transcription factor PPARɣ directly suppresses heparanase transcription by inhibiting its promoter activity. These results demonstrate that PPARɣ agonists, like pioglitazone, reduce proteinuria

by attenuating glomerular heparanase expression via transcriptional regulation. These results provide novel insight in the potential mechanism of the renoprotective effects of the PPARɣ agonists, i.e. thiazolidinediones, in diabetes mellitus patients with renal disease and non-diabetic renal disease patients [22-24].

Uniform flow protects the endothelium via mechanotransduction, which results in the subsequent activation of essential signalling pathways in endothelial cells [25-29], including MAPK7 signalling. Activation of MAPK7 signalling suppresses endothelial dysfunction and endothelial-to-mesenchymal transition (EndMT). Conversely, the loss of MAPK7 signalling facilitates EndMT [30]. EndMT plays a pivotal role in the formation of neointimal lesions in atherosclerosis [31]. In our lab, it was previously shown that uniform flow reduces the expression of histone methyltransferase EZH2, and that repression of EZH2 reciprocally activates MAPK7 signalling [32]. In **chapter 6**, studies on the reciprocal cross-talk between MAPK7 and EZH2 in endothelial cells and the process of EndMT are described. We showed that MAPK7 activation induces silencing of EZH2 via the expression of microRNA (miR)-101. EZH2 silences the miR-200 family, resulting in increased expression levels of the phosphatases DUSP1 and DUSP6. DUSP1 and DUSP6 in their turn dephosphorylate MAPK7 resulting in the inhibition of MAPK7 activity. In lysates of total human atherosclerotic coronary arteries, the expression of EZH2 and DUSP1 is increased and the expression of MAPK7 is decreased. In human umbilical vein endothelial cells *in vitro*, the ectopic expression of miR-101/-200a/-429 restored the balance between MAPK7 activity and EZH2, and prevented the process of EndMT. This study shows that a delicate balance in the reciprocal signaling between MAPK7 and EZH2 is pivotal for the prevention of EndMT. Disturbances in this reciprocal signalling circuit associate with the induction of EndMT and the severity of human coronary atherosclerosis.

CONCLUSIONS

In this thesis, we identified epigenetic, transcriptional and translational mechanisms, with a focus on EZH2, PPARɣ, and MAPK7, that modulate endothelial gene transcription and contribute to endothelial dysfunction in glomerulosclerosis and atherosclerosis. We show that proper regulation of endothelial gene transcription is of utmost importance to maintain functional endothelial cells. PPARɣ is already used as therapeutic target in renal disease patients and in this thesis we show that the renoprotective effects of PPARɣ agonists can be explained by transcriptional regulation of HPSE gene expression. We revealed that increased EZH2 expression and activity induces a loss of the endothelial

glycocalyx by modulation of expression of endothelial glycocalyx-associated genes. Finally, we demonstrated that reciprocal cross-talk between EZH2 and MAPK7 and differential activity of both EZH2 and MAPK7 induces EndMT. We therefore conclude that EZH2 and MAPK7 pose potential efficacious targets to restore endothelial dysfunction and halt disease development and progression in glomerulosclerosis and atherosclerosis. A schematic representation of the investigated involvement of EZH2, PPARɣ, and MAPK7 in endothelial dysfunction is depicted in figure 1.

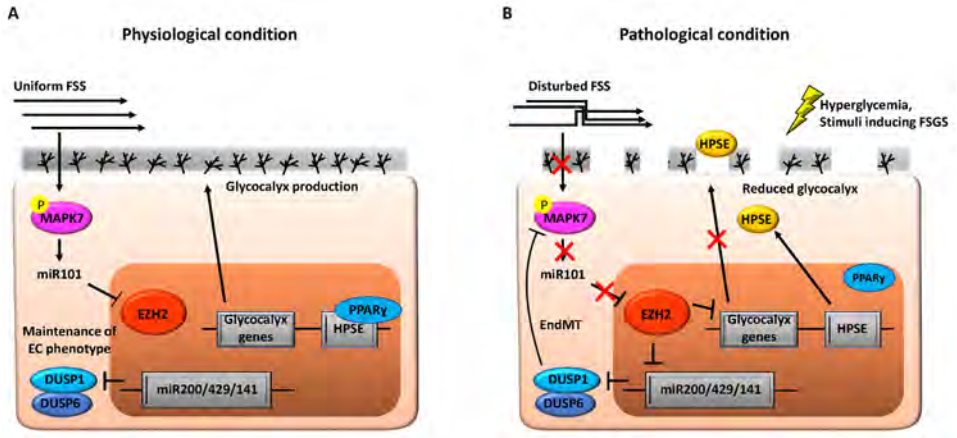

Figure 1. Schematic representation of the involvement of EZH2, PPARɣ and MAPK7 in endothelial dysfunction. (A) Under physiological conditions in endothelial cells, uniform fluid shear stress (FSS) induces the phosphorylation of Mitogen-Activated Protein Kinase 7 (MAPK7) in endothelial cells, resulting in microRNA-101 (miR101) gene expression and a decrease in expression of Enhancer of Zeste Homolog 2 (EZH2), hereby preserving the endothelial cell phenotype. Low levels of EZH2 and the EZH2-mediated histone modification trimethylation of lysine 27 of histone 3 (H3K27me3) allow expression of glycocalyx-associated genes resulting, in maintenance of the endothelial glycocalyx. The transcription factor peroxisome proliferator-activated receptor gamma (PPARɣ) binds to the promoter of the heparan sulphate degrading enzyme heparanase (HPSE), thereby preventing HPSE expression. (B) Upon disturbed FSS, MAPK7 activation and signalling is lost, resulting in reduced miR-101 expression. Loss of miR-101 results in decreased repression of EZH2, resulting in an increase in EZH2 expression. Increased EZH2 expression causes a reduction of miR-200/-429/-141 expression via epigenetic repression through H3K27me3. Under physiological conditions, these miRs reduce the expression of dual specificity protein phosphatase (DUSP) 1 and DUSP6. Decreased expression of these miRs results in an increased expression of DUSP1 and DUSP6. Increased expression of DUSP1 and DUSP6 results in the conservation of the disturbed FSS-induced loss of MAPK7 activation, and eventually the process of EndMT. Upon other noxious stimuli, i.e. hyperglycemia, the expression of EZH2 and the presence of H3K27me3 increase, culminating in transcriptional inhibition of glycocalyx-associated gene expression resulting in a compromised endothelial glycocalyx. Upon stimuli that induce FSGS, PPARɣ binding to the HPSE promoter is reduced, resulting in increased expression levels of HPSE and a reduction of the endothelial glycocalyx by cleavage of heparan sulphate by HPSE.

TRANSLATIONAL ASPECTS AND FUTURE PERSPECTIVES

Aberrant gene expression patterns largely contribute to GEnC dysfunction. Altered epigenetic mechanisms seem to be causally involved in modulating aberrant gene expression in GEnC. Until now, the knowledge of the epigenetic mechanisms involved in GEnC dysfunction in DN and FSGS was scarce and needed to be expanded. We show that Enhancer of Zeste Homolog 2 (EZH2)-mediated epigenetic modification trimethylation of lysine 27 of histone 3 (H3K27me3) is increased in GEnC in DN, thereby indirectly reducing the glomerular endothelial glycocalyx, which is an important aspect of the pathogenesis of DN. Recently, transcriptome analysis of GEnC isolated from DN mice revealed that epigenetic enzymes, involved in DNA methylation and histone modifications, are differentially expressed in GEnC from DN mice compared to GEnC from healthy mice [33]. Interestingly, the expression of the histone demethylase lysine-specific demethylase 6A (KDM6a) was downregulated in GEnC in DN [33]. KDM6a specifically demethylates H3K27me3 and removes EZH2-mediated H3K27me3 [34]. These results suggest a reduced removal of methyl groups from H3K27, resulting in increased H3K27me3 presence in GEnC in DN, which is in line with our findings of increased H3K27me3 presence in GEnC in mouse and human DN. Our finding of the increased levels of H3K27me3 in GEnC in DN mthus be the result of an increased EZH2-mediated methylation of H3K27 and/or of a decreased KDM6a-mediated demethylation of H3K27me3, both possibilities not being mutually exclusive and potentially co-existing.

Decreasing H3K27me3 in GEnC could offer a new therapeutic strategy for the treatment of DN. We analysed H3K27me3 presence solely in GEnC in mouse and human kidney samples and not in other glomerular cell types including podocytes. In contrast to our findings of increased H3K27me3 presence in GEnC, H3K27me3 was recently shown to be decreased in podocytes in DN. Decreased podocyte H3K27me3 correlates with the extent of podocyte damage due to activation of Notch signalling and loss of quiescence [35]. From the therapeutic perspective, pharmacological inhibition of H3K27me3 may lead to lower levels of H3K27me3, which may be detrimental for podocytes. Therefore, cell-specific interventions are needed when therapeutically targeting H3K27me3 in GEnC to prevent the development or progression of glomerulosclerosis.

To investigate whether H3K27me3 could be used as potential new therapeutic target in DN, we utilized a cell-targeting approach by the E-selectin targeted delivery of SAINT-O-Somes (SOS) loaded with siRNA specific for EZH2 (siEZH2) to GEnC in DN. E-selectin-targeted SOS loaded with siEZH2 (SOS-siEZH2) can successfully be delivered to the

glomerular endothelium. A trend towards decreased H3K27me3 presence was shown, but a reduction of EZH2 could not be revealed. In siSCR- and siEZH2-SOS-treated BTBR$^{ob/ob}$ mice, glomerular EZH2 mRNA expression was hardly detectable using quantitative Real-Time PCR (qRT-PCR) (data not shown), suggesting that EZH2 mRNA expression levels may have been too low to allow a further reduction. The low EZH2 mRNA expression was confirmed by bright field *in situ* hybridization experiments that we performed on renal tissue from 24-week old BTBR$^{ob/ob}$ mice. An explanation for the absence of a reduction in EZH2 and H3K27me3 *in vivo* could be that the SOS did not release its cargo (siEZH2) in BTBR$^{ob/ob}$ GEnC. However, this is highly unlikely, since E-selectin-targeted SOS loaded with siRNA have previously been shown to effectively deliver siRNA into activated GEnC *in vivo* and to decrease the target mRNA expression preventing disease progression [18, 19]. The only difference in targeting strategy between the current and previous [18, 19] studies is the specificity of the siRNA used. Although the current EZH2-specific siRNA induced a 71% reduction of EZH2 mRNA expression in mouse endothelial cells *in vitro*, the siEZH2 may not have reduced EZH2 expression to an effective extent in GEnC *in vivo*. Although our *in vivo* data revealed decreased glomerular collagen I expression and an increased glomerular perfusion area after siEZH2-SOS treatment, a reduction in EZH2 could not be shown and therefore it is still elusive whether reduced EZH2-mediated H3K27me3 in GEnC is beneficial in glomerulosclerosis in DN. This needs to be investigated in future research, in which endothelial cell-specific EZH2 knockout mice suffering from DN could be instrumental. To this end, EZH2$^{lox/lox}$ mice [36] and inducible VE-cadherin-*Cre* mice [37] could be mated to generate inducible endothelial cell-specific EZH2 knockout mice in which the development of DN could be investigated. In addition to our findings that EZH2-mediated H3K27me3 indirectly reduces the glomerular endothelial glycocalyx, chromatin immunoprecipitation sequencing (ChIP-Seq) in GEnC with high and low levels of EZH2-mediated H3K27me3 is needed in order to reveal all H3K27me3-regulated genes in GEnC, by which increased EZH2-mediated H3K27me3 presence in GEnC may contribute to the pathogenesis of glomerulosclerosis in DN. As previously stated, our finding of the increased levels of H3K27me3 in GEnC in DN may be the result of an increased EZH2-mediated methylation of H3K27 and/or of a decreased KDM6a-mediated demethylation of H3K27me3. Since glomerular EZH2 mRNA expression was hardly detectable in our BTBR$^{ob/ob}$ mouse model and since we could not show a reduction in EZH2 in GEnC after targeting of EZH2, increased H3K27me3 levels could also be the result of decreased KDM6a-mediated demethylation of H3K27me3. It should therefore, by immunofluorescence staining, be investigated whether KDM6a protein expression is decreased in GEnC in renal tissues of BTBR$^{ob/ob}$ mice as well as renal tissues of DN patients. If so, GEnC-specific liposomal delivery of KDM6a mRNA could offer a therapeutic strategy to increase KDM6a expression and thereby decrease H3K27me3 presence in GEnC in DN.

It is currently unknown which stimuli are responsible for the increased H3K27me3 presence in GEnC in DN. High glucose levels increase EZH2 in retinal microvascular endothelial cells [38]. Exposure of GEnC to high glucose is therefore a likely explanation for the increase in EZH2-mediated H3K27me3 in DN. To study this, we stimulated GEnC with high glucose or methylglyoxal (MGO), a radical produced upon high glucose stimulation and a major precursor of advanced glycation endproducts (AGEs), and investigated its effects on EZH2 expression and H3K27me3 presence. In these preliminary analyses the effects of high glucose on EZH2 and H3K27me3 were inconclusive due to inconsistent results, but exposure of GEnC to MGO did induce an increase in H3K27me3 presence and EZH2 mRNA expression. An excess of MGO increases the production of reactive oxygen species and causes oxidative stress [39], suggesting that oxidative stress in DN may culminate in increased levels of H3K27me3.

We describe in our literature review (CH2) that GEnC dysfunction is pivotal in the early development of both DN and FSGS, and that these pathologies share common pathogenetic mechanisms. Our findings on increased EZH2-mediated H3K27me3 in DN still needs to be investigated in experimental and clinical FSGS in order to determine a common role for EZH2 in DN and FSGS. Next to EZH2-mediated H3K27me3, the identification of other epigenetic modifications inducing aberrant gene expression in GEnC in DN and FSGS is of utmost importance to obtain a better understanding of the pathogenetic pathways involved in GEnC dysfunction in these pathologies. Combining transcriptome profiling of GEnCs with data from epigenomic databases, such as encyclopedia of DNA elements (ENCODE) in which epigenetic modifications are mapped in various cell lines [40], could reveal additional epigenetic modifications responsible for aberrant expression patterns in GEnC. CRISPR-Cas9 technology would be a promising future therapeutic tool in DN, to alter expression of target genes in GEnC by epigenetic editing. Using the d(ead)CAS9 platform, histone modifying enzymes can be recruited to a specific locus to add or remove epigenetic modifications locally. Hereby target genes can be inhibited or re-expressed [41] to intervene in aberrant gene expression inducing GEnC dysfunction. As noted before, cell-specific delivery is needed to therapeutically intervene in GEnC to avoid detrimental off-target cell effects. Cell-specific delivery of CRISPR-Cas is still a huge challenge since the CRISPR-Cas9 system consists of multiple components which all need to be packaged and delivered in order to achieve cell-specific delivery of the CRISP-Cas9 system [41]. Since the expression of epigenetic enzymes can influence the amount of epigenetic modifications, delivery of siRNA to decrease and delivery of mRNA to increase transcripts encoding or regulating the expression of epigenetic enzymes holds great potential to interfere with epigenetic modifications in GEnCs.

Next to the involvement of altered epigenetic mechanisms, we show altered transcriptional mechanisms to be involved in modulating gene expression in GEnC as well. Glomerular expression of HPSE, a key glycocalyx-degrading enzyme, is increased in many glomerular diseases [5, 17]. HPSE is responsible for decreasing heparan sulphate (HS) and associates with the development of proteinuria [5, 17, 20, 21]. In this thesis, we show that the PPARɣ agonist pioglitazone reduces proteinuria in experimental FSGS, in part by inhibiting glomerular HPSE expression via transcriptional repression as was demonstrated in both GEnC and podocytes *in vitro*. PPARɣ potentially inhibits HPSE expression via co-repressors such as the nuclear receptor corepressor (NCoR) or the silencing mediator of retinoid and thyroid hormone receptors (SMRT) via histone deacetylases [42]. Next to experimental FSGS, HPSE is previously shown to be key in the development of experimental DN and experimental glomerulonephritis [5, 17]. PPARɣ agonists are being used in clinical practice for several decades, mainly as treatment of insulin resistance in type 2 diabetes. The effects of the PPARɣ agonist rosiglitazone have been investigated in FSGS patients. Rosiglitazone was shown to be effective in FSGS based on stabilization of the estimated glomerular filtration rate (eGFR) [43]. Next to DN, PPARɣ agonists could therefore provide therapeutic potential in FSGS as well.

Lastly, we show that EZH2 and MAPK7 together form a reciprocal signalling circuit involving epigenetic, translational and post-translational regulation in endothelial cells. Disturbed reciprocal signalling between EZH2 and MAPK7 induces EndMT *in vitro.* EndMT is important in the formation of neointimal lesions in atherosclerosis [31]. We show that the expression levels of EZH2 and MAPK7 are increased and decreased respectively in atherosclerotic plaques of human coronary arteries, suggesting the involvement of disturbed signalling between EZH2 and MAPK7 in atherosclerotic plaque formation. We did not investigate the pathways being activated upon disturbed reciprocal signalling between EZH2 and MAPK7, resulting in EndMT. Activation of Tumor Growth Factor β (TGFβ) signalling would be a plausible explanation for the induction of EndMT upon disturbed signalling between MAPK7 and EZH2. TGFβ signalling is a known inducer of EndMT and MAPK7 activation indirectly represses canonical TGFβ signalling via SMAD7 [44, 45]. Additionally, inhibition of EZH2 inhibits TGFβ-signalling [46]. A decrease in MAPK7 activity and an increase in EZH2, as observed in the disturbed signalling between MAPK7 and EZH2, would therefore induce TGFβ signalling. Previous research showed that inhibition of MAPK7 signalling by miR-374b in endothelial cells induces EndMT [47], and endothelial-specific deletion of MAPK7 exacerbates atherosclerosis [48]. Furthermore, methylation of H3K27 is increased in early as well as advanced stages of human atherosclerotic plaques [49]. These results strengthen our results of the involvement of disturbed MAPK7 and EZH2 activity in

atherosclerosis. Therefore, *in vivo* restoration of the reciprocal signalling between EZH2 and MAPK7 possibly exerts protective effects and provides a new therapeutic strategy in atherosclerosis. Endothelial cell-specific inhibition of EZH2, for example by targeted delivery of siRNA against EZH2, is a potential efficacious therapeutic strategy to prevent aberrant gene expression, endothelial dysfunction and the development and progression of atherosclerosis. Currently, there are no ongoing clinical trials investigating the effects of reducing EZH2 in atherosclerosis. However, at the moment several clinical trials are running with the selective small molecule inhibitor of EZH2, tazemetostat (also known as EPZ-6438), in cancer patients. Cancer and its treatments are known to be able to induce the development of atherosclerosis [50, 51], and EZH2 activity is associated with cancer progression [52]. The currently ongoing clinical trials include a phase I clinical trial in patients with chordoma, a rare type of cancer of the spine [53] and a phase I clinical trial in patients suffering from relapsed or refractory B-cell non-Hodgkin lymphoma or an advanced solid tumour [54]. Such phase I studies have cardiovascular outcome parameters included as an indicator of safety and tolerability of EZH2 inhibition. Access to these data could provide further study to gain new insights in the effectiveness of EZH2 inhibition on cardiovascular function.

CONCLUDING REMARKS

In conclusion, our findings elucidate the importance of epigenetic, transcriptional and translational mechanisms, involving EZH2, PPARγ, and MAPK7, in the modulation of endothelial gene transcription and in the induction of endothelial dysfunction in glomerulosclerosis and atherosclerosis. We show that changes in only one epigenetic, transcriptional or translational mechanism in endothelial cells can have a great impact on endothelial function and might have a fundamental role in the development of vascular pathologies in humans. The consequences of changes in epigenetic, transcriptional and translational mechanisms should not be underestimated and restoration of these mechanisms should be considered as a therapeutic strategy in vascular pathologies. PPARγ is already used as therapeutic target in renal disease patients. Before considering EZH2-mediated H3K27me3 as a new therapeutic target for the treatment of glomerulosclerosis in DN patients, a reduction in H3K27me3 presence in GEnC needs to be achieved in experimental DN and its consequences on GEnC dysfunction and renal function need to be clear. Restoration of the reciprocal signalling between EZH2 and MAPK7 and its consequences on the formation of neointimal lesions should be evaluated in experimental atherosclerosis first, before implementing the restoration of reciprocal signalling between EZH2 and MAPK7 as a new therapeutic strategy in atherosclerotic patients.

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